

A MORPHOLOGICAL AND FUNCTIONAL STUDY OF THE VENOM APPARATUS IN REPRESENTATIVES OF SOME MAJOR GROUPS OF HYMENOPTERA

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Summary

An analysis is made of the structure of the venom apparatus, or the more primitive organs by which it may be represented, in 17 species, principally of Australian origin, belonging to the major hymenopterous groups Tenthredinoidea and Siricoidea (Symphyta), Ichneumonoidea (Apocrita-Parasitica), Scelioidea, Vespoidea, Formicoidea, and Apoidea (Apocrita-Aculeata).

Consideration is given to the relationship between the venom apparatus of more advanced Hymenoptera and the accessory reproductive glands of other groups of insects. An important link between the two is found to be provided by the Symphyta.

Evidence is presented of evolutionary continuity from group to group of Hymenoptera in the relationship of the gland filaments synthesizing venom-type secretion to the reservoir storing it; in the development and degeneration of the musculature of the reservoir wall; and in the relative positions and structure of the endings of the exit ducts from glands belonging to the system. Major changes of form in components of the apparatus are related to what is known of changes in their functions.

I. INTRODUCTION

Nineteenth-century morphological studies on the female reproductive system of insects first directed attention to the characteristic way in which, within the Hymenoptera, certain components of the system are specialized to act as a venom apparatus. In its fully developed form the basic elements of this apparatus (Bernard 1951) are a simple or multiple gland structure secreting venom, a reservoir in which the venom is stored, and an associated gland, Dufour's gland, sometimes also called the "alkaline", or the "accessory" gland, whose function has only been conjectured, except in one or two specialized cases (Wilson 1963). The major part of the apparatus is concentrated in the posterior region of the hymenopterous gaster, with the reservoir in some cases extending well forward. Ducts from the venom reservoir and Dufour's gland pass back into a chitinous sting, to the base of which is sometimes applied a pair of "sting" glands (Snodgrass 1956; Ghent and Gary 1962) [(that is, "Koschevnikov's" glands (Wilson 1965)], and through which the secretion of the apparatus is ejected.

The venom apparatus, as such, is closely associated with other organs of the female reproductive system, some of which are also glandular. Of these, one or two pairs of glandular pouches (James 1926; Grandi 1929) may be developed to supply

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the median vagina (= uterus), which is confluent with the united oviducts from the ovaries of the two sides. In addition, spermathecal glands (Snodgrass 1935), whose function is related to the action of sperms during fertilization of eggs descending to the gonopore, may be allied with the spermatheca, which also opens into the vagina. In the Hymenoptera such organs have sometimes been misidentified as glandular components of the venom apparatus, although they are normally different in their morphological relationships as well as in their functions.

At the present time increasing attention is being directed to problems concerning the structure and evolution of the venom apparatus as a result of investigations in progress on the chemical, toxicological, and behavioural characteristics of its glandular secretions (Cavill and Robertson 1965). Only Bordas (1895) has so far studied the general morphology of the venom apparatus in a representative series of groups of Hymenoptera. But he failed to consider the phylogenetic significance of the findings. The current work aims to extend knowledge of the apparatus to a number of additional hymenopterous species, and to aspects of structure not previously considered, while attempting to obtain some indication of the overall pattern by which the venom apparatus may have evolved within the Hymenoptera.

II. REVIEW

(a) Relationship of the Hymenopterous Venom Apparatus to Elements of the Reproductive System in other Insects

In attempting to build up a pattern by which the venom apparatus may have evolved, we must look first for its origin to the female reproductive system before specialization, as it is known to occur in other groups of insects.

Glands like those of the venom apparatus of the Hymenoptera, which are accessory* to the female reproductive system, occur in the majority of insect orders (Grassé 1949, Vol. 9, 1951, Vol. 10; Imms 1956). In the primitive Thysanura, for example, the gonads retain a metameric arrangement, but are united in a terminal vagina with a single opening in the ninth abdominal segment. Accessory glands are developed in relation to the vagina, but open independently of it. In the seventh segment of the Odonata, in which the reproductive system is also primitive, the oviducts open into a spermatheca which is supplied by accessory glands. Paired accessory, here called colleterial, glands open into the vagina of the Dictyoptera, whose gonopore lies between the seventh and eighth abdominal segments. In the tettigonioid Orthoptera special glands open between the valves of the ovipositor, an organ which originates from the eighth and ninth abdominal sternites. In the Acridoidea and at least one family of Coleoptera accessory glands are attached to the lateral oviducts, whose origin is mesodermal. But in the Trichoptera and Heteroptera they supply the common oviduct,† which is ectodermal. Accessory glands occur most usually in relation to the tubular vagina,‡ which is confluent with the common oviduct, or in relation to its counterpart, an open genital chamber.‡

* The term "accessory gland" is a general one which applies to any glandular area associated with the terminal part of the reproductive system.

† These terms are used in the sense of Snodgrass (1935).

This condition is found in the Plecoptera, Isoptera, Planipennia, Mecoptera, some Coleoptera, Diptera, Lepidoptera, and Homoptera.

According to Berlese (1909), during early larval instars of insects genital invaginations are formed in the seventh, eighth, and ninth sterna of the abdomen, from which the common oviduct, the spermatheca, and the accessory glands develop. But Berlese did not think it possible to establish homologies from group to group, on the grounds that these structures may differ in their number and origin. George (1928), working on the Homoptera, tended to support this view.

Christophers (1923) and Christophers and Barraud (1926) showed that in the Diptera the common oviduct develops from an invagination of the eighth abdominal segment, the spermatheca from a second invagination of the same segment, and the accessory glands from a ninth segment invagination. About the same time, Pruthi (1924) found that the common oviduct was invaginated from the eighth sternum, and both spermatheca and accessory glands from the ninth, in the Coleoptera. Nel (1930) found comparative anatomical evidence to suggest the development of accessory genital invaginations from the eighth and ninth sterna in the Heteroptera, Thysanoptera, Isoptera, Trichoptera, Neuroptera, Mecoptera, Mallophaga, Lepidoptera, and Hymenoptera. On this basis, and in the light of his own investigations on the Dictyoptera, Orthoptera, and Dermaptera, he concluded that accessory reproductive structures originate from invaginations of the eighth and ninth sterna as an ancestral condition of all insects.

In the Diptera the three independent invaginations of the common oviduct, the spermatheca, and the accessory glands later in development are approximated to open into a common genital chamber (Christophers 1923; Christophers and Barraud 1926). A similar approximation of the common oviducal and spermathecal invaginations into a tubular vagina is recognized in the Hymenoptera (D'Rozario 1942). Snodgrass (1935) pointed out that while the opening of the common oviduct, the gonopore, through which eggs are expelled, may lie within the genital chamber, the genital chamber itself may serve as a copulatory pouch whose external opening is the vulva.

As further modifications, one or more of the three primary invaginations may be lost during development, or the original pattern may be masked by the formation of secondary invaginations (Nel 1930). In the latter way develop such glands as those earlier referred to, formed in association with the lateral oviducts, the common oviduct, the spermatheca, and the ovipositor.

In respect to the Hymenoptera, D'Rozario (1942) confirmed Nel's views, which were based on comparative anatomy, by a study of development. He showed that buds of the genitalia appear on the eighth and ninth abdominal sterna of third-instar larvae in the Symphyta, and of the earliest larval instar in the more highly evolved Apocrita. The pair of buds on the eighth sternum become the ventral valves of the ovipositor, or sting, the outer pair on the ninth become the dorsal valves, and the remaining pair the inner valves. The spermatheca is invaginated from the bases of the ventral valves of the eighth sternum, and the venom gland,

or glands, from the bases of the inner valves of the ninth. A more anterior, median invagination of the ninth sternum gives rise to Dufour's gland.

Here, then, is evolutionary continuity between the venom apparatus of the Hymenoptera and elements of the reproductive system of other insects. The chitinous components of the sting are derived directly from the ovipositor. In addition, the venom and Dufour's glands which supply the sting are linked in their ninth segmental origin with the female accessory reproductive glands formed in orders ranging from the primitive Thysanura to the highly evolved Coleoptera, Lepidoptera, and Diptera. In these groups glands of the ninth segment open either into the genital chamber or the dorsal region of the vagina. They tend to be paired, in conformity with the ancestral pattern of the insect reproductive system (Gustafson 1950). They may be tubular and simple, or branched, or each expanded into a reservoir. The glands of each side may open separately, or unite in a common duct with a single opening (Grassé 1949, 1951). Their secretions normally relate to oviposition, and may be oily or mucous. They may form a protective layer over the egg as it is laid, cement the egg to the substrate on which it is deposited, or form a hard case enclosing a batch of eggs during development (Snodgrass 1935). It will be of interest to consider further how the adult form and function of the hymenopterous venom apparatus may reflect its link in origin with female accessory reproductive structures such as these.

(b) Form of the Venom Apparatus in the Hymenoptera

Studies on the hymenopterous venom apparatus carried out up to the beginning of the present century were broad in scope, and extended to 9 of the 17 currently accepted superfamilies (Cavill and Robertson 1965) making up the order. Apart from investigating variations in the general structure of the apparatus (Bordas 1895), particular attention was paid to the gland now known by the name of Dufour (1841), who first described it; to the development of the sting (Dewitz 1875, 1877; Zander 1899); and to the detailed morphology of the apparatus in highly evolved Formicidae (Forel 1878; Janet 1902). Subsequent investigations tended, in themselves, to be of more restricted scope, but added three more to the range of superfamilies examined. All these earlier studies of the venom apparatus can best be analysed further in relation to the major groups of Hymenoptera considered in phylogenetic sequence.

(i) Symphyta

Elements of the female accessory reproductive gland complex of this most primitive group of Hymenoptera have long been known, but little attempt has been made to relate them to the venom apparatus of more advanced groups. Sebific and serific accessory reproductive glands were figured in the female of *Tenthredo succincta* (Tenthredinoidea: Tenthredinidae) (Dufour 1841). Two branching gland filaments, a non-muscular reservoir, and an additional, terminally enlarged gland were recorded in *Emphytus tibialis* and *E. cinctus* (Tenthredinidae) (Bordas 1895). In *Orussus sayii*, of the rare family Orussidae (Orussoidea), Cooper (1953) described a ventrally placed, median gland supplied by numerous gland filaments, and with a single, tubular organ originating near its base. But only D'Rozario (1942)

suggested that the single female accessory reproductive gland of *Nematus ribesii* (Tenthredinidae), from its mode of origin, might be homologous with the venom glands of higher Hymenoptera.

(ii) *Apocrita-Parasitica*

The venom apparatus proper is recognized first in this group, in which (for example in *Ichneumon* (Ichneumonoidea: Ichneumonidae)) there are from 8 to 10 gland filaments, with several primary stems originating from the apex of the venom reservoir. An originally double vaginal gland pouch is developed in addition to the venom and Dufour's glands in the *Ichneumon*, *Pimpla*, *Tryphon*, and *Ophion* groups of Ichneumonidae (Pampel 1914). There are a similar number of venom-gland filaments in *Habrobracon juglandis* (Braconidae), but they are related to the base, not the apex, of a reservoir whose wall is strongly thickened by muscles, including bands of longitudinal muscles (Bender 1943). The venom-gland filaments are enlarged and branched in *Polemon liparae* (Braconidae) (D'Rozario 1942), while they are reduced to two which are unbranched in the braconid *Lysiphlebus fabarum*. Tremblay (1964) showed in the latter that the reservoir contains a spiral pump which, on expansion, draws fresh liquid from the filaments to replace expended secretion.

In the pteromalid *Pteromalus liparae* (Chalcidoidea) there are a pair of vaginal gland pouches in addition to a greatly reduced venom reservoir and Dufour's gland. Two unbranched gland filaments enter the reservoir, which is muscular, in the phytophagous chalcid *Harmolita* (Eurytomidae) (James 1926). *Euchalcidia caryobori* has a very thin-walled reservoir and, for the first time, only one venom-gland filament (Hanna 1934).

(iii) *Apocrita-Aculeata*

Multiple venom-gland filaments occur again in the Sphecoidea. For example, in *Cerceris arenaria* and *C. variabilis* (Crabronidae) 10-15 tubules radiate out from each of two widely separated basal filaments. A third gland occurs in both the Crabroninae and Philanthinae (Bordas 1895). In *Pompilus* and *Priocnemis* (Pompiloidea) the two primary venom gland filaments divide into several branches, which in turn bifurcate (Bordas 1895). The two gland filaments remain unbranched in *Vespa germanica* (Vespoidea) and enter the reservoir independently at the apex. Here the development of muscles in the reservoir wall is carried still further than in the Braconidae, with heavy, regularly arranged muscle strands running obliquely between base and apex, and divided by four longitudinal furrows (Bordas 1895).

In some species of Andrenidae (Apoidea) the venom reservoir has a muscular wall, but in the majority of groups of Apoidea it is non-muscular. There are no more than two unbranched gland filaments, which tend to unite, in their region of entry into the reservoir, in a median arm which may be short in *Bombus muscorum* or extended in *Apis mellifera*. In certain Andrenidae and Halictidae Dufour's gland is greatly enlarged and the venom gland reduced in size (Bordas 1895). Within the Apidae, Kerr and de Lello (1962) were able to demonstrate evolutionary stages in the degeneration of the venom apparatus, from the primitive, well-developed condition in *Psithyrus*, through intermediates in *Bombus*, to genera of "stingless bees"

(Meliponini) like *Trigona* and *Melipona*, in which Dufour's gland disappears completely and the venom reservoir may be vestigial or absent.

Differences in the form of the venom apparatus are particularly striking between subgroups of Formicoidea. In the primitive bull ant, *Myrmecia gulosa* (Myrmeciinae), it follows the generalized pattern, seen also in primitive wasps and bees, of two gland filaments, a muscular-walled, spheroidal reservoir and a smaller, tubular Dufour's gland (Cavill, Robertson, and Whitfield 1964). Whelden (1957a, 1957b, 1960) may have misinterpreted some of the structures of the venom gland complex he found in the primitive Ponerinae, but Hermann and Blum (1966) showed the complex in *Paraponera clavata* to be close to that of *Myrmecia*. Blum and Callahan (1963) found characteristics linking the primitive with the higher ant subfamilies in the venom apparatus of the Pseudomyrmicinae.

Earlier knowledge of the formicoid venom apparatus was based on Forel's (1878) studies of highly evolved subfamilies. He found a bourreleted type system in the Myrmicinae and Dolichoderinae, and a pulvinate system in the Formicinae, depending on the relationship of the gland filaments to the venom reservoir. In *Solenopsis saevissima*, an example of the bourreleted type, Callahan, Blum, and Walker (1959) described what appears to be a filter through which the poison passes into the reservoir, and noted that in the honey bee Dufour's gland as well as the venom duct open into the sting bulb, a matter of controversy. All components of the venom apparatus in the Dolichoderinae, which are described in detail by Pavan and Ronchetti (1955), are rudimentary, and are superseded by anal glands (Emery 1912).

In the Formicinae the stinging mechanism has evolved to a stage of degeneration, although the venom glands and reservoir remain highly developed (Beyer 1890). Here, as in the Apoidea prior to the reduction and loss of the venom apparatus, Dufour's gland assumes additional importance, becoming spherical in *Lasius*, and bilobed instead of tubular in *Formica* (Forel 1878) and *Camponotus* (Forbes 1938).

(c) Function of Venom Apparatus Components

Far less is known of the function of components of the venom apparatus than of their structure and, up to the present, basic differences of function have been recorded only in the social insects. In most of the Symphyta and the more primitive Apocrita ninth segmental accessory glands retain the reproductive functions known for glands in similar positions in other insect groups, such as secreting a protective coating for the egg and lubricating it during its passage down the ovipositor, to which is now added the more highly evolved function of preparing a substrate for the developing larva.

Bender (1943) thought that a gland opening on the vagina in *Habrobracon juglandis* (Apocrita: Braconidae) must function as an egg lubricator. Tremblay (1964) described a gland of similar function opening into the ovipositor of *Lysiphlebus fabarum* (Braconidae). James (1926) suggested that secretions from what can be regarded as the venom apparatus proper in the phytophagous chalcid *Harmolita* might initiate, if not entirely cause, the process of gall formation. In *Blastophaga psenes*, a chalcid of similar feeding habits, the secretions appear to influence multiplication

of secondary nuclei in the embryo sacs of its host plants, leading to parthenogenetic development of the endosperm which serves as larval food (Grandi 1920).

The majority of Parasitica use the venom apparatus to paralyse or kill the other insects on, or in, which the eggs are laid and the larvae develop, but many Aculeata employ it as a means of killing the prey (Bernard 1951) they carry away as food for their brood. Components of the venom apparatus are diverted to entirely new functions in certain Aculeata such as members of the Myrmicinae and Formicinae (Formicoidea). For example, the true venom gland secretes the odour trail material in *Atta texana* (Myrmicinae) (Moser and Blum 1963), while Dufour's gland secretion produces an alarm reaction in *Camponotus* (Formicinae) (Maschwitz 1964) and acts as an odour trail in *Solenopsis saevissima* (Myrmicinae) (Wilson 1959).

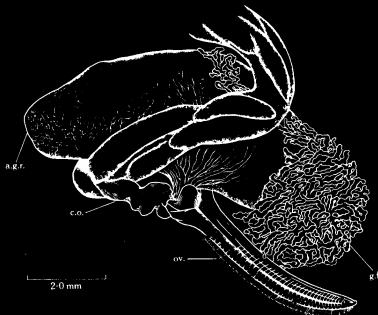


Fig. 1.—Female accessory reproductive glands of *Perga affinis* Kirby (Tenthredinoidea: Tenthredinidae) in relation to reproductive system. a.g.r., reservoir; c.o., common oviduct; g.f., gland filaments; ov., ovipositor.

III. VENOM APPARATUS STRUCTURE IN MAJOR GROUPS OF HYMENOPTERA*

Further representatives of the Hymenoptera, the majority of them Australian, were examined to produce additional information on evolutionary continuity in the form of the venom apparatus from group to group, and on the correlation of changes in form with changes in function.

Living material was anaesthetized with carbon dioxide, dissected in insect Ringer's solution (Roeder 1953, p. 161; solution of Yeager 1939), stained in aqueous anilin blue plus orange G, and fixed in 1% formaldehyde in Ringer's solution. Some of the material was dissected after preservation in 4% formaldehyde, or in 70%

* See Cavill and Robertson (1965, Fig. 2).

ethanol. A few preparations were rapidly dehydrated after fixation and mounted whole in Euparal. The majority of illustrations were prepared from stained dissections held in Ringer's solution plus formaldehyde, and were drawn with a camera lucida attachment to a stereoscopic microscope.

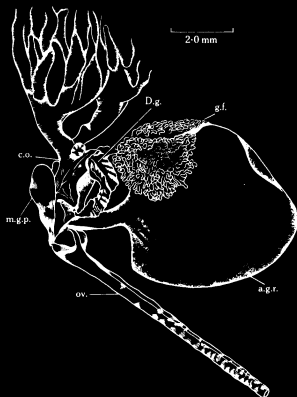


Fig. 2.—Female accessory reproductive glands of *Sirex noctilio* L. (Siricoidea: Siricidae) in relation to reproductive system. *D.g.*, Dufour's gland; *m.g.p.*, mycelial gland pouch.

1. Symphyta

The females of this suborder, with the possible exception of the superfamily Orussoidea, use the ovipositor and its associated glands in the process of laying eggs in plant tissue, a link with orders such as the Coleoptera, Lepidoptera, and Hemiptera. This function has not brought about the development of a venom apparatus of the characteristic form recognized in those Hymenoptera preying on other insects. But the symphyte accessory reproductive gland complex is of particular significance as a basic structure from which the venom apparatus of higher forms may have evolved.

(a) *Tenthredinoidea*

Perga affinis Kirby (Tenthredinidae) (Figs. 1, 14, 26)

This Australian sawfly has the ovaries and the paired oviducts extending dorsally to the posterior end of the body, then turning forward and ventrad to

unite in a common oviduct (Fig. 1, *c.o.*) which is enlarged into a vagina opening at the base of the ovipositor. An accessory reproductive gland structure lies between the arms of the paired oviducts in the region where they are directed forward. It consists of glandular filaments (*g.f.*), a median reservoir (*a.g.r.*) storing their secretion, and an exit duct running into the ovipositor (*ov.*). The filaments are very numerous. They branch and anastomose, are 1.5–7 or 8 mm in length, and vary in width from 0.05 to 0.13 mm. The majority open into a median, tubular extension of the posterior apex of the reservoir, the remainder entering by a number of irregularly arranged side tubes (Fig. 14). The reservoir fills the greater part of the body

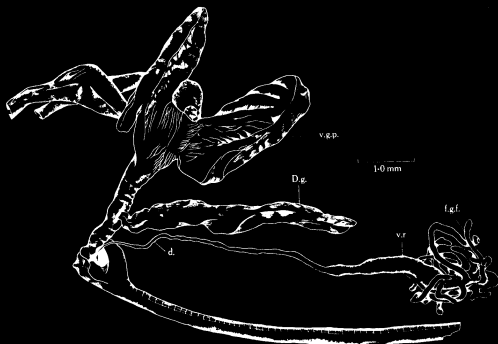
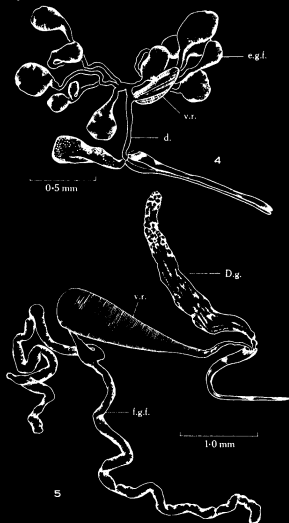


Fig. 3.—Accessory reproductive and venom glands of *Megarhyssa nortoni nortoni* (Cresson) (Ichneumonidae: Ichneumonidae) in relation to reproductive system. *f.g.f.*, free venom gland filaments; *v.r.*, venom reservoir; *d.*, venom duct; *v.g.p.*, vaginal gland pouches.

cavity in the posterior region of the gaster, measuring 6–9 mm long, by approximately 4 mm in depth, and 3.5–4.5 mm in thickness (Fig. 1) in *P. affinis* specimens of 11–16 mm total body length. Its contents are of mucilaginous consistency. The reservoir wall is supported by a network of interlacing muscle bands, of which the strongest bands converge towards the exit duct. The exit duct is short, wide, and opens to the exterior (Fig. 26, *a.g.d.o.*) immediately behind the vaginal opening (*e.o.*). The posterior lip of the opening is formed by a thickened half-loop of tissue (*c.m.*) with a rectangular, chitinous plate centrally, which acts as a stopper for the mouth of the duct. The soft membrane behind it is cleft into two phlanges (*e.g.*) lying at right angles to the stopper, and normally recessed into the base of the ovipositor. When the ovipositor is flexed during the passage of an egg, the posterior

lip will be drawn away from the soft anterior lip, the phlanges acting as egg guides, so that each egg issuing from the vaginal pore will be bathed in a drop of secretion released from the opened mouth of the duct.



Figs. 4 and 5.—Venom apparatus of Braconinae sp. (Ichneumonoidea: Braconidae) (4) and *Tachynomyia adusta* (F. Sm.) (Scolioidea: Thynnidae) (5). *e.g.f.*, enlarged venom gland filaments.

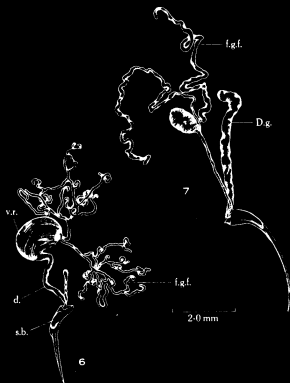
D'Rozario (1942) described a somewhat similar gland structure to that of *Perga* in the tenthredinid *Nematus*, but in the latter the reservoir wall lacks muscular support.

(b) *Siricoidea*

Sirex noctilio L. (Siricidae) (Figs. 2, 15, 27)

The ovaries extend back only to the level of the base of the ovipositor, then pass vertically downwards into the oviducts, which unite and expand into a thick-

walled vagina, receiving a median spermatheca. Along the two sides of the vagina, and supported by the lancets of the ovipositor, run mycelium-bearing pouches (Fig. 2, *m.g.p.*). A single, tubular gland (*D.g.*) opens posteriorly midway along the vagina. Its wall is thin and folded, and it narrows slightly at the base to a slit-like opening. Its secretion is oily, not mucilaginous, and it is here regarded as the most primitive example of the sebific gland described by Dufour in 1841, and now known as Dufour's gland. Immediately posterior to Dufour's gland is an accessory reproductive gland which consists of numerous gland filaments (*g.f.*) divided into two



Figs. 6 and 7.—Venom apparatus of *Diamma bicolor* Westwood (Scoliidea: Thynnidae) (6) and *Campsomeris (Dielis) tasmaniensis* Sauss. (Scoliidea: Scoliidae) (7). *s.b.*, sting bulb.

masses of approximately equal size, a large median reservoir (*a.g.r.*), and an exit duct. The filament masses are based on a pair of tubular outgrowths of the anterior apex of the reservoir (Fig. 15), each approximately one-quarter the width of the reservoir. The basal tube narrows rapidly, is bent back on itself, and gives off a number of thick, primary filaments. These in turn produce the numerous bifurcating side branches, 0.06–0.08 mm in diameter, which make up the bulk of each filament mass. Secretory gland tissue is confined to the branching filaments and does not penetrate into the basal tube.

The gland reservoir extends from behind the ovaries to near the posterior end of the body. It is laterally compressed, measuring approximately 4.5 mm long,

by 3·7–4 mm in depth, and 3·5 mm in thickness (Fig. 2) in specimens of *S. noctilio* of 12–15 mm total body length. Its contents are mucilaginous, but its wall lacks muscular strengthening. The exit duct from the reservoir originates about the centre of the anterior face. It passes forward, turns back at an acute angle into the base of the ovipositor, and opens at some distance from the vaginal pore. At the bend of the duct a heavy muscle (Fig. 27, *s.m.*) runs transversely across its ventral surface, closing the duct when the ovipositor is in normal retracted position. But when the ovipositor is flexed the muscle draws the ventral wall from the dorsal, and permits secretion to issue from the duct. The duct opening is supported by a narrow half-loop of chitin, and terminates in a pair of chitinous phlanges acting as egg guides.

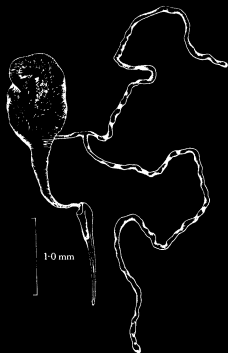


Fig. 8.—Venom apparatus of *Bothroponera* sp. (*?porcata* F. Sm.) (Formicidae: Ponerinae). Dufour's gland could not be traced in the few specimens available for study.

Buchner (1953) described the mycelium-bearing pouches of *Sirex* and other Siricidae, and recognized them as the source of the fungus lining larval tunnels. Burmeister (1836) wrongly considered that the gum (i.e. mucilage) coating the eggs of *Sirex* was produced by Dufour's gland. But the gland immediately posterior to Dufour's gland, which corresponds to the accessory reproductive gland described in *Perga* in the preceding section, is almost undoubtedly the "gum gland" Burmeister sought. It differs from that of *Perga* in the absence of muscular strengthening in the reservoir wall, the development of an exit duct which is considerably longer, but of smaller diameter, and the subdivision of the irregularly arranged gland filaments into two equal masses.

2. Apocrita

The venom apparatus of this second subdivision of the Hymenoptera is linked with the accessory reproductive glands of the Symphyta in the interrelationship of its parts and the regions supplied by its secretions, although its general form is strikingly different.

(a) *Parasitica* : *Ichneumonoidea*

(i) *Rhyssa persuasoria* L. (Ichneumonidae) (Fig. 28)

There is no clearly demarcated venom reservoir, the region being represented by only a slight and gradual enlargement of the terminal part of the venom duct. Two or three asymmetrically placed venom gland filaments, dividing into 5–10 sub-branches, join the reservoir region on each side at its apex. The walls of the venom reservoir and duct are non-muscular, but slightly folded transversely. A single lobe of tissue, which probably represents the fused egg guide phlanges (*e.g.*) of the

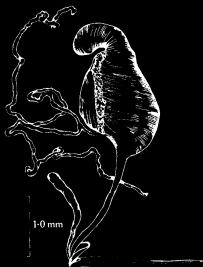


Fig. 9.—Venom apparatus of *Leptogenys sjostedji* F. Sm. (Formicidae: Ponerinae).

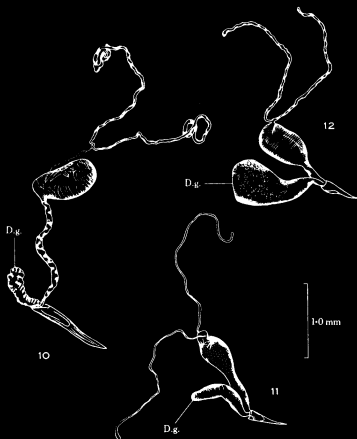
Symphyta, is inserted into the sting bulb, and the venom duct is extended along it to open near its tip (Fig. 28, *v.d.o.*). A tubular Dufour's gland occupies the same relative position as that of *Sirex*, opening into the wall of the vagina before it reaches the base of the sting.

Bordas (1917) illustrated the venom reservoir of *R. persuasoria* as an enlarged sac clearly separated off from the venom duct, but no evidence for the development of such a structure has been found in the present study.

(ii) *Megarhyssa nortoni nortoni* (Cresson) (Ichneumonidae) (Figs. 3, 16)

A pair of large gland pouches (Fig. 3, *v.g.p.*) and a small median spermatheca open into the vagina near its junction with the common oviduct, and a tubular Dufour's gland enters towards its base. The venom glands proper are a series of

filaments (*f.g.f.*) opening at the sides of the zone representing the venom reservoir (*v.r.*), which is the enlarged end of the venom duct. There are two primary filaments on each side, branching dichotomously into up to six secondary branches (Fig. 16). Glandular tissue lines a central cavity within the filaments, but is not invaginated into the reservoir. The reservoir wall is formed of a layer of cuticle thrown into irregular folds, but devoid of muscles. The reservoir narrows gradually into the



Figs. 10-12.—Venom apparatus of *Rhytidoponera* sp. [*?aciculata* (F. Sm.)] (Formicidae: Ponerinae) (10), *Podomyrma* sp. [*?gratiosa* F. Sm.] (Formicidae: Myrmicinae) (11), and *Aphaenogaster longiceps* F. Sm. (Formicidae: Myrmicinae) (12).

venom duct (Fig. 3, *d.*), which becomes very slender indeed as it passes into the sting bulb. A long, thin lobe of tissue, which is continuous with the wall of the vagina, runs into the sting bulb. It terminates in two minute, chitinous flaps which, in position and relationships, represent vestiges of the egg guides of the Symphyta. The venom duct, instead of ending outside the sting bulb, is extended along the membranous lobe almost to its tip, where the opening is strengthened by a chitinous rim. Although Dufour's gland, as it passes towards the sting bulb, is associated

with the venom duct, Dufour's gland opening in the vaginal wall is far removed from the opening of the venom duct within the sting.

The venom apparatus of *Megarhyssa* takes on a closely similar form to that of *Rhyssa*, except that the gland filaments, instead of originating at the distal end of the reservoir zone, arise some distance from it.

(iii) *Subfamily Braconinae*, gen. et sp. indet. (Braconidae)* (Figs. 4, 17)

Here the venom gland filaments are directly associated with the base rather than the apex of the reservoir. About 12 filaments (Fig. 4, *e.g.f.*) each terminating in a globular enlargement, and originating from two or three primary branches on each side, are united near the distal end of the venom duct. The duct is bent at the point of junction with the filaments, before passing into a cone-shaped reservoir (*v.r.*). The reservoir is clearly separated off from the remainder of the venom duct,

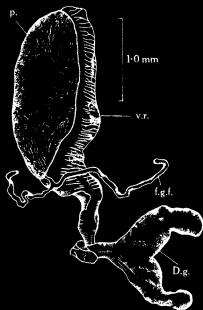
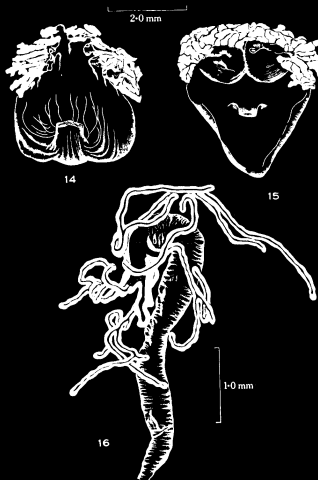


Fig. 13.—Venom apparatus of *Camponotus* sp. No. 1 (ANIC) (Formicidae: Formicinae), showing bilobed Dufour's gland. *p.*, pulvinus.

and is enveloped in a thick layer of longitudinal muscles. Beneath the muscle layer the cuticular reservoir wall is thrown into dense, regular, transverse folds. Gland tissue lines the terminal enlargements of the filaments but does not extend into the reservoir. Instead, a valvular structure (Fig. 17) controls the entry into the reservoir of secretion from the gland filaments. Contraction of the longitudinal muscles of the reservoir wall will drive secretion down into the sting. Relaxation of the muscles will extend the concertina wall of the reservoir, and draw fresh secretion from the filaments through the valve into the reservoir. In this species, Dufour's gland, which

* The Commonwealth Institute of Entomology states that it has not been possible to identify this braconid species. Some characters would place it in the Doryctini, but the absence of certain significant features may indicate a closer relationship with the Rogadini. Specimens have been deposited in the British Museum, Cromwell Road, London, S.W.7, and in the Australian National Insect Collection, Canberra. Date of collection of specimens, 4.i.1960; locality, Sydney.

is approximately the same length as the venom duct, is not uniformly tubular, but enlarges gradually into a terminal sac. The species is one of the earliest forms whose Dufour's duct, instead of opening into the wall of the vagina, passes with the venom duct into the base of the sting and opens beside it.

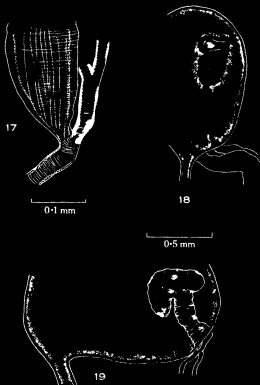


Figs. 14-16.—14, Entry tubes of gland filaments in relation to posterior face of accessory reproductive gland reservoir of *Perga affinis*. 15, Anterior face of accessory reproductive gland reservoir of *Sirex noctilio*. 16, Venom reservoir of *Megarhyssa nortoni nortoni*.

The basal junction of the gland filaments with the reservoir is a direct progression from their intermediate position in *Megarhyssa*, just as the close-set, transverse folding of the reservoir wall is a further development of the irregular folding in *Megarhyssa*. It is likely that the wall folding in the present species of braconid represents the spiral pump described by Tremblay (1964) in *Lysiphlebus fabarum*.

(b) *Aculeata* : *Vespoidea**Polistes humilis* Fabr. (Vespidae)

The venom reservoir, placed posteriorly in the upper right side of the gaster, is spheroidal and comparatively large, with a slender duct of approximately its own length. Up to five-sixths of the volume of the reservoir is made up of a layer of muscles. They are transverse externally, but towards the inner part of the layer are turned to run longitudinally. The reservoir is marked on the outside by four evenly spaced,

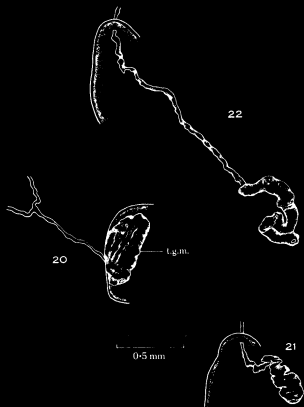


Figs. 17-19.—17, Junction of duct from gland filaments with venom reservoir in *Braconinae* sp. 18, 19, Invagination of ducts from gland filaments and terminal gland tissue into venom reservoir in *Campsomeris tasmaniensis* (18) and *Diamma bicolor* (19).

longitudinal furrows which are the lines of attachment of the muscles to the underlying layer of the wall. This cuticular layer, the tunica propria of the reservoir, is raised into four corresponding ridges. Two very slender, unbranched gland filaments pass independently into the apex of the reservoir. Within the reservoir wall the two filaments combine in a common arm which is expanded into a phlange of glandular tissue invaginated into the cavity of the reservoir. Dufour's gland is tubular, its most common form through the Hymenoptera, and approximately the same length as the venom duct. It narrows slightly at the base and terminates on the outside of the lobe of tissue inserted into the sting bulb, at the level of the margin of

the sting. The venom duct extends to the tip of the lobe, where its opening is supported by thickened cuticle.

The invagination of a phlange of glandular tissue into the venom reservoir replaces the valve mechanism at the junction of gland filament and reservoir in the preceding braconid species. Its development represents a major advance in the evolution of the venom apparatus.



Figs. 20-22.—Invagination of terminal venom gland tissue into venom reservoir of *Rhytidoponera* sp. (?*aciculata*) (20), *Podomyrma* sp. (?*gratiosa*) (21), and *Aphaenogaster longiceps* (22). t.g.m., terminal mass of venom gland tissue.

(c) *Aculeata* : *Scoliioidea*

(i) *Diamma bicolor* Westwood (Thynnidae) (Figs. 6, 19, 29)

This Australian flower wasp has a venom reservoir bent at right angles to the axis of the venom duct. The wall is strengthened by heavy muscles running longitudinally from base to apex of the reservoir, the longitudinal strands anastomosing at intervals. There are two free gland filaments entering the reservoir independently, but close together, at the apex. Each filament branches dichotomously at intervals along its length, and normally forms a tangled mass in the region of the reservoir.

Within the reservoir the two filaments are united in a mass of gland tissue, which is attached to the reservoir wall only at the point of entry (Fig. 19). The tissue mass has a basal stalk whose wall is transversely folded, and passes into a shoe-shaped, distal expansion, at the heel of which the ducts from the two filaments open separately into the cavity of the reservoir. Dufour's gland is tubular, but small and weakly developed. It terminates in a slit-like, raised opening on the phlange of tissue inserted into the sting bulb, at about the level of the margin of the bulb (Fig. 29; *D.d.o.*). The venom duct opening (*v.d.o.*) is large, but the thickening of the rim is similar in form to that of *Rhyssa*.

(ii) *Tachynomia adusta* (F. Smith) (Thynnidae) (Figs. 5, 32)

The venom reservoir (Fig. 5, *v.r.*), which is an elongated, pear-shaped sac 1.5 mm in length, increasing in diameter from 0.12 mm at the base to 0.5 mm at the apex, lies in a gaster which is half as long again by three times as wide. The cuticular reservoir wall is enveloped in a single thin layer of transverse muscles, which tend to anastomose, but do not form longitudinal bands. The venom gland filaments (*f.g.f.*) are large, but reduced to only two in number which are unbranched. They are approximately 3.25 mm long and of a uniform thickness of 0.09–0.11 mm. They are united into a short basal filament of 0.1–0.13 mm long, which enters the reservoir at one side of its apex, but is not invaginated into the reservoir cavity. Dufour's gland (*D.g.*) is from two-thirds as long, to the same length, as the venom reservoir plus its duct. But its greatest width is no more than 0.23–0.24 mm at about the centre, from which it tapers to 0.08–0.11 mm towards the base and apex. Its surface is nodulated, the nodules tending to run in longitudinal bands, which are most conspicuous in the apical third. Large masses of gland tissue (Fig. 32; *g.t.m.*) are developed laterally in relation to the triangular plates of the ovipositor, and an area (*t.m.*) of the intersegmental membrane supporting them is thickened into longitudinal ridges.

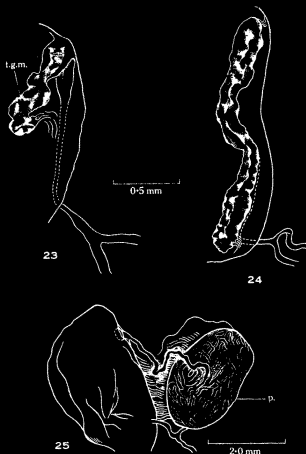
The gland tissue masses associated with the triangular plates are at least homologous with the sting glands previously recognized in *Apis mellifera*, and now discovered also in *Myrmecia gulosa*, *Rhytidoponera ?aciculata*, and *Bothroponera ?porcata* (Formicoidea) (see below).

(iii) *Campsomeris* (*Dielis*) *tasmaniensis* Sauss. (Scoliidae) (Figs. 7, 18)

Here the venom reservoir has a strongly muscular wall, with transverse, not longitudinal, muscle strands. The pattern of musculature appears to be directed towards exerting the maximum control over the terminal area of venom gland tissue, which enters the reservoir laterally towards its base. In *Campsomeris* there are two very long, unbranched venom gland filaments passing independently into the reservoir. The basal diameter of the filaments is up to one-third that of the reservoir, and there is some indication that one filament is larger than the other. Within the venom reservoir, the mass of gland tissue terminating the filaments takes the form of a flattened, rectangular thickening of the wall (Fig. 18). Dufour's gland is tubular, but very large, extending beyond the venom reservoir, where it is bent at right angles. Two lateral masses of gland tissue and one ventral are enormously enlarged, and are

related to the basal plates of the sting in the positions normally taken by the sting gland complex.

Since the Scoliidæ are not social insects, the glandular masses associated with the basal plates of the sting may be involved in some reproductive function. They may produce a secretion acting as a sex attractant, or ensuring that the sting does not contaminate the tissue into which it penetrates, or that the host is not attacked by other insects.



Figs. 23–25.—Common venom gland filament running between outer and inner layers of reservoir wall, and terminating in mass of gland tissue invaginated into venom reservoir, in *Bothroponera* sp. (?*porcata*) (23) and *Leptogenys sjostedji* (24), and convoluted to form a pad (pulvinus) between outer and inner layers of reservoir wall in *Camponotus* sp. No. 1 (25).

(d) *Aculeata* : *Formicoidea*

Currently, the divergent forms taken by the venom apparatus in more highly developed subfamilies of Formicidæ have been re-examined in the light of the

structure of the apparatus in Australian representative of the primitive Ponerinae and Myrmeciinae.

- (i) *Rhytidoponera* sp., close to *aciculata** (F. Sm.) (Formicidae: Ponerinae) (Figs. 10, 20, 33)

The two venom gland filaments are long, slender, unbranched, and united in an elongated basal arm which originates laterally at about one-third of the distance from the posterior end of the reservoir. Only a short length of filament is incorporated in the reservoir wall, before it passes to the large, glandular cushion (Fig. 20, *t.g.m.*) within the reservoir. The reservoir wall is completely encompassed by a network of fine, interlaced muscle fibres, ensuring direct control over expansion and contraction of the sac. Dufour's gland (Fig. 10, *D.g.*) is short and considerably thicker than in other primitive ants, and its yellowish contents can be seen through the transparent wall of the proximal part of the sac. Sting glands (Fig. 33, *s.g.*) are developed in *Rhytidoponera* towards the region of the triangular plate, where they are attached to the intersegmental membrane.

There is a change in position in the sting glands of *Rhytidoponera* as compared with homologous glands related to the quadrate plate in the honey bee (Snodgrass 1925; Ghent and Gary 1962) and the ponerine ant *Stigmatomma pallipes* (Whelden 1957).

- (ii) *Bothroponera* sp. (?*porcata* F. Sm.) (Formicidae: Ponerinae) (Figs. 8, 23)

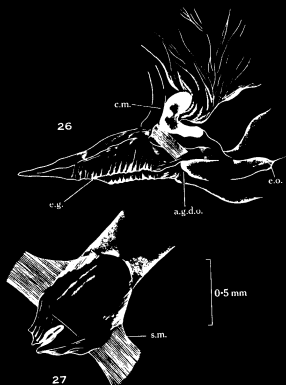
The venom gland filaments have the same form as those of *Rhytidoponera* ?*aciculata*, but the basal arm enters the reservoir wall nearer the posterior end of the sac. The venom reservoir is spheroidal, with an interlaced network of muscle fibres in the wall, which tend to radiate out from the internally developed terminal gland mass. The basal filament arm runs between the outer and inner layers of the reservoir wall almost from base to apex, where it expands into a long, irregularly shaped lobe (Fig. 23, *t.g.m.*) hanging in the cavity of the reservoir. Dufour's gland is short, slender, and weakly developed. Sting glands are well developed, extending in the intersegmental membrane from the region of the triangular plate along the margin of the quadrate to the spiracular plate.

- (iii) *Leptogenys sjostedji* F. Sm. (Formicidae: Ponerinae) (Figs. 9, 24)

The venom reservoir (Fig. 9), unlike that of other species so far recorded, is enlarged at the base, and drawn out into an elongated, curved lobe extending to the anterior end of the gaster. The venom gland filaments are unbranched, and united in a common arm which penetrates the reservoir at its base. This arm runs forward inside the reservoir wall to the anterior lobe, where it passes into a longitudinally doubled band of gland tissue, which extends back to the point of entry of the gland filament arm, applied to the reservoir wall throughout its length (Fig. 24). The muscles of the reservoir wall form close-set transverse bands between which no anastomosis has been detected. Their direction is slightly oblique to the long axis of the reservoir, but at right angles to the gland region, permitting the maximum

* Material cannot be referred to the species with certainty in view of current systematic problems, in this and the following genus.

effect of contraction and expansion to be exerted on it. The duct from the reservoir to the sting approximately equals the length of the reservoir. Dufour's gland is tubular, slightly distended distally, and extends a little beyond the base of the venom reservoir. The sting glands are small, and appear as two aggregations of gland cells dorsal to the triangular plates of the sting.

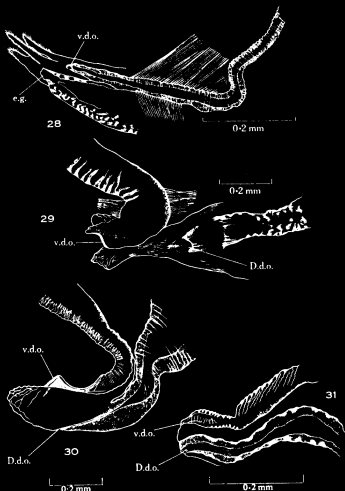


Figs. 26 and 27.—Termination of exit duct from female accessory reproductive gland reservoir in *Perga affinis* (26), showing closure mechanism bearing central chitinous plate, horseshoe-shaped opening immediately posterior to egg opening, and egg guides dissected from base of ovipositor; termination of exit duct from female accessory reproductive gland reservoir in *Sirex noctilio* (27). *a.g.d.o.*, accessory gland duct opening; *c.m.*, closure mechanism of accessory gland duct; *e.g.*, egg guides; *e.o.*, egg opening; *s.m.*, support muscle for accessory gland duct opening.

(iv) *Myrmecia gulosa* Fabr. (Formicidae: Myrmeciinae) (Figs. 30, 34)

The general characteristics of the venom apparatus of this species have already been described (Cavill, Robertson, and Whitfield 1964) but the following additional points are relevant to the present study. The single, basal arm from the venom gland filaments runs between the muscular and cuticular layers of the reservoir wall, from its entry point about one-third of the distance from the base of the reservoir to the corresponding point near its apex. Here the terminal invaginated gland tissue forms

an oval mass constricted off from the reservoir wall. The constriction is carried further in *Myrmecia pyriformis*, where the gland tissue forms a mushroom-shaped body within the reservoir. The venom duct of *M. gulosa*, running from the reservoir to the sting, terminates near the tip of the tissue lobe within the sting bulb (Fig. 30).



Figs. 28-31.—Phlange of tissue dissected from sting bulb, showing vestigial egg guides and opening of venom duct in *Rhyssa persuasoria* (28), and openings of venom duct and Dufour's duct in *Diamma bicolor* (29), *Myrmecia gulosa* (30), and *Aphaenogaster longiceps* (31). D.d.o., Dufour's duct opening; v.d.o., venom duct opening.

Its raised opening is supported by chitinous thickening, and, in the retracted position of the sting, closure of the duct is effected by pressure of its thin side against its thick. The lumen of Dufour's duct is narrowed to a minute opening at approximately the same level as that of the venom duct. A branch of the same nerve as controls muscles of the venom reservoir wall controls muscles of the wall of Dufour's gland.

and provides evidence that Dufour's gland is involved directly in the process of venom ejection. The sting glands of *M. gulosa* are two well-formed masses of gland cells, each cell attached to the intersegmental membrane in the region of the triangular plate by a long, simple, cuticular duct.

(v) *Podomyrma* sp. (?gratiosa F. Sm.) (Formicidae: Myrmicinae) (Figs. 11, 21)

The two venom gland filaments (Fig. 11) are slender and unbranched, but united basally to form a "Y", their common arm penetrating the venom reservoir at its apex. The venom reservoir is spheroidal with a cuticular wall, most of it devoid of an outer muscle layer, but with a few anastomosing muscle strands surrounding the point of entry of the gland filament. The common arm of the gland filaments is not bound into the wall after it penetrates the reservoir, as in the lower ants, but passes directly into a terminal mass of gland tissue. This tissue forms an irregularly shaped, tapering lobe suspended inside the cavity of the reservoir (Fig. 21). Dufour's gland, which is smaller than the venom reservoir, forms a short lobe rather than an elongated tube, as in the lower ants. No sting gland cell masses have been found in the regions of the quadrate or triangular plates.

In *Podomyrma* ?gratiosa the yellow and oily appearance of the contents of Dufour's gland is in contrast to its whitish, opaque appearance in the Myrmecinae.

(vi) *Aphaenogaster longiceps* F. Sm. (Formicidae: Myrmicinae) (Figs. 12, 22)

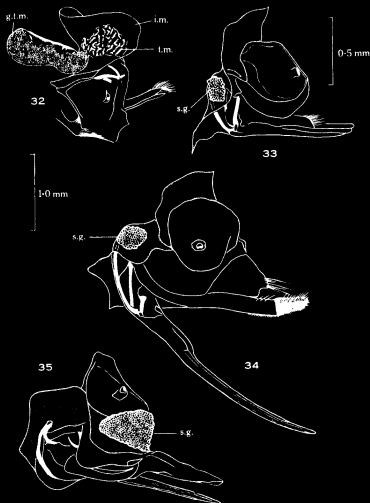
The pair of venom gland filaments (Fig. 12) are about 1.5 times as long as the venom reservoir, and they are united in a short common arm attached to the reservoir slightly to one side of its apex. The major part of the reservoir wall is supplied by transverse and longitudinal muscle strands, but these are weakly formed, irregular and broken at intervals. Inside the venom reservoir the common gland filament continues as a very slender tube for about twice the length of the reservoir. It is free from the walls, but forms a tightly coiled mass in the apex of the cavity. It passes rather suddenly into an enlarged terminal length of tube about as long as the reservoir, but coiled to half its length (Fig. 22). This species is the only member of the Formicoidea examined in the course of the present work in which Dufour's gland (Fig. 12, *D.g.*) exceeds the venom reservoir in size. It no longer takes the form of a tubular phlange, but is made up of a basal duct expanded into a spheroidal sac, similar to the venom reservoir. The sac is 1½ times as long as the venom reservoir and twice the diameter. Its duct is approximately the same length as the venom duct, but twice as wide. The wall of Dufour's sac is cuticular and rather tough, with an embossed surface. No sting gland cell masses have been found.

(vii) *Camponotus* sp. No. 1 (ANIC)* (Formicidae: Formicinae) (Figs. 13, 25)

The two venom gland filaments (Fig. 13; *f.g.f.*) are short and slender, and unite at the base of the venom reservoir. Instead of being invaginated into the reservoir, the common gland filament passes into an expanded cushion (*p.* = pulvinus, hence "pulvinate") lying between the outer and inner layers of the reservoir wall. The cushion has an extremely thin, cuticular covering extending over the duct from the

* The present state of the systematics of the Australian section of this genus is such that it is necessary to hold species on record in the Australian National Insect Collection (ANIC) by number.

gland filament, which remains slender and unbranched, but is convoluted and bound into a dense mass forming the body of the cushion (Fig. 25). Ultimately this duct, without enlarging, empties into the reservoir underneath the centre of the cushion.



Figs. 32-35.—Gland tissue mass and thickened membrane in region of triangular plate of ovipositor of *Tachynomyia adusta* (Scolioidea : Thynnidae) (32). Sting gland in region of triangular plate of sting in *Rhytidoponera* sp. (?*aciculata*) (Formicidae : Ponerinae) (33), *Myrmecia gulosa* (Formicidae : Myrmeciinae) (34), and in region of quadrate plate of sting in *Apis mellifera* (Apoidea : Apidae) (35). *g.t.m.*, gland tissue mass; *i.m.*, normal, thin intersegmental membrane; *t.m.*, thickened intersegmental membrane; *s.g.*, sting gland.

In specimens dissected immediately after being anaesthetized the cushion appears like a silver plate, suggesting that either the convoluted duct or the space surrounding it is filled with air. Interlaced muscle strands support the wall of the reservoir, which passes into a short, broad exit duct, about one-quarter as long as the reservoir.

No sting glands appear to be developed, just as no functional sting is formed, the sting sclerites being reduced to rudiments. The quadrate, oblong, triangular, and spiracular plates are represented by minute rods supporting the vestibule into which the venom and Dufour's gland ducts open independently. Here, as in other Formicinae, it appears that the secretion from the venom reservoir is ejected from the vestibule as a spray, probably mixed with air expelled through the spiracular openings of the sting, which are well developed despite the degeneration of the remainder of the apparatus.

(e) *Aculeata* : *Apoidea*

Apis mellifera L. (Apidae) (Fig. 35)

Some characteristics of the venom apparatus of this species have been re-examined, since more recent studies have failed to agree with earlier findings. Dissection shows that Dufour's duct does not open into the sting chamber below the sting, as Kerr and de Lello (1962) suggest it does on the evidence of sections, but that both Dufour's duct and the venom duct penetrate the sting bulb, as in other highly evolved Aculeata. The mass of gland cells developed in association with the quadrate plate of the sting, and together forming a sting gland (Fig. 35, *s.g.*) which Maschwitz (1964) failed to find, have here been demonstrated without difficulty.

IV. DISCUSSION

(a) *Morphological Evolution of the Venom Apparatus*

Sufficient material has been studied to indicate the general direction of the morphological changes undergone from group to group by the venom reservoir and the venom gland filaments (Fig. 36).

The shape of the reservoir varies from the asymmetrical sac, compressed laterally and elongated anteroposteriorly, which is its forerunner in *Perga* and *Sirex*, to a zone scarcely differentiated from the venom duct in *Rhyssa* and *Megarhyssa*, a symmetrical, spindle-shaped structure in a species of Braconinae, and a spheroid in higher groups.

There is little muscular strengthening of the reservoir wall in groups more primitive than the Braconidae. But in the section of Aculeata leading from the Scoliidae and Thynnidae to the Vespidae, the muscle layer becomes increasingly well developed until it occupies up to five-sixths the volume of the reservoir. It decreases again to a network of interlacing strands in primitive Formicidae such as the Myrmeciinae and Ponerinae. The reduction is carried still further in the Myrmicinae and Formicinae, and the musculature of the wall is lost completely in the Dolichoderinae, as it is in the advanced Apidae.

The originally large number of gland filaments of the Symphyta, arising by a process of dichotomous branching, is gradually reduced to two basal filaments of increasing length, through the Parasitica and more primitive Aculeata, to the Formicoidea and Apoidea. Although there is a tendency to dichotomous branching throughout the Hymenoptera, it is fully realized only in primitive forms up to

Braconinae, in *Diamma* (Thynnidae), and according to Bordas (1895), some Sphecoidea and Pompiloidea, but in higher forms no more than minute nodules appear on the basal filaments.

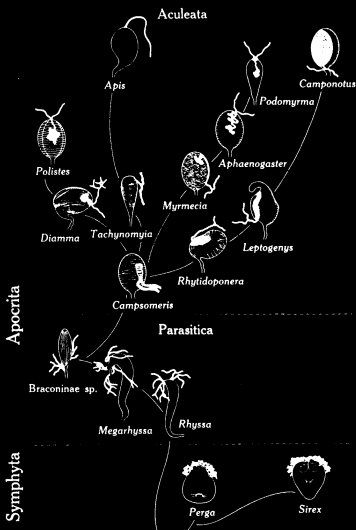


Fig. 36.—Diagram of the evolution of the venom gland tissue and venom reservoir in currently studied genera of Hymenoptera, representing the Tenthredinoidea, Siricoidea, Ichneumonoidea, Scoliidea, Vespoidea, Formicoidea, and Apoidea.

In primitive Hymenoptera the filaments join the reservoir at its apex, the point of junction is lowered in *Megarhyssa*, and in a species of Braconinae they join the venom duct at a point below the base of the reservoir. But the change of position is not maintained in the Aculeata, and reverts to an apical junction in some Vespidae, advanced Formicidae, and Apidae.

The relationship of gland tissue to reservoir is of particular phylogenetic significance, although it has been little considered up to the present. In the Symphyta and Parasitica secretory tissue lines the gland filaments, but does not extend into the reservoir. But the valvular mechanism in Braconinae foreshadows the pattern followed in the Aculeata, in which the terminal tissue of the basal filament is invaginated into the venom reservoir. In primitive genera of Formicidae the basal filament runs between the muscular and cuticular layers of the reservoir wall, before terminating in a mass of tissue applied to the wall internally near the apex. The tissue mass becomes progressively more elongated in *Rhytidoponera*, *Bothroponera*, and *Leptogenys* (Ponerinae). But in *Aphaenogaster* and *Podomyrma* (Myrmicinae), in which the muscle layer surrounding the reservoir is reduced or absent, the basal filament penetrates the wall directly and ends in a phlange covered by the cuticular lining of the reservoir, but hanging freely in its cavity. This agrees with the structure described for representatives of the Myrmicinae by Forel (1878) and Callahan, Blum, and Walker (1959), and indicates a break between Ponerinae and Myrmicinae in the evolution of the form taken by the invaginated tissue. No such break is apparent between its form in the Ponerinae as a flattened pad, in which the basal arm from the gland filaments is bent back once on itself, and the cushion of tissue (pulvinus) in the Formicinae, whose mass is made up of the greatly extended, convoluted basal arm. At the same time, the mushroom-shaped structure recorded during the present work in *Myrmecia pyriformis* F. Sm. and other Myrmeciinae, with its freely hanging, enlarged tip and narrow stalk, is clearly ancestral to the structure confirmed here for the Myrmicinae. These direct links between Ponerinae and Formicinae, Myrmeciinae and Myrmicinae, are contrary to the views of Brown (1954) and Eisner and Brown (1958) on the phylogeny of the Formicidae, based on the structure of the proventriculus. With regard to the venom apparatus, therefore, group relationships may perhaps be better expressed according to Bernard's (1951) plan, in which there is no deeply cleft subdivision into poneroid and myrmecoid complexes, and the principal evolutionary line proceeds more or less directly through to the Formicinae.

The formation of the opening of the exit duct from the reservoir has a bearing on the question of homology between the accessory reproductive glands of the Tenthredinoidea and Siricoidea and the venom glands of the higher Hymenoptera. In *Perga* (Tenthredinoidea) the opening of the exit duct is strengthened posteriorly by a chitinous bow against which the unstrengthened part remains effectively shut except during the passage of an egg from the genital chamber to the ovipositor. The egg is supported by a pair of egg guides posterior to the duct opening. In *Sirex* (Siricoidea) the duct runs forward and is bent back on itself as it passes to the ovipositor. A heavy, transverse muscle within the loop tends to constrict the duct when the ovipositor is in normal, retracted position, while a pair of egg guides position the egg in relation to the gland opening when the ovipositor is flexed. The transverse loop muscle of *Sirex* is repeated beyond the Symphyta in *Rhyssa* (Apocrita-Parasitica). This phylogenetic link between Symphyta and Parasitica is emphasized in *Rhyssa* by the formation within the ovipositor of a small lobe of tissue lying beneath the end of the venom duct, which may represent fused, degenerate egg guides. Moreover, the venom duct opening is supported by a chitinous bow against

which the thin-walled part of the duct is held shut except during flexing of the sting. The repetition of this closure mechanism supports the view that the venom glands of the Aculeata are derived from accessory reproductive glands allied to those of the Symphyta.

(b) *Coordination of Form with Function*

It is important to consider the significance, in terms of function, of the structural changes in the so-called venom apparatus which have been traced through the Hymenoptera. Certainly there is little direct experimental evidence of function on which to develop such an analysis, but some guide to it is provided by group habits.

(i) *Venom Glands*

In the first place, the accessory female reproductive gland filaments and reservoir of the Tenthredinoidea and Siricoidea are directly related structurally to the ninth segmental glands of orders such as the Coleoptera, Lepidoptera, and Diptera [cf. Section III(a)]. Moreover, the mucilage which has been observed to coat the eggs when they are laid in plant tissue (Chrystal 1928) is clearly produced by this gland complex. The complex therefore appears to agree in its function of protecting the eggs, as well as in its segmental origin and its form, with the corresponding glands of other insect groups.

The striking change in form from the accessory reproductive glands of the Tenthredinoidea and Siricoidea (Symphyta) to those of the Orussoidea (Cooper 1953) accompanies a major break in habit from laying eggs in plants to laying them on, or in association with, insect hosts. The form taken by the gland filaments and reservoir in *Orussus* is repeated in the Parasitica, in *Rhyssa* and *Megarhyssa*. It signals the appearance of the venom apparatus proper, for in the latter genera the venom gland secretion is thought to paralyse the prey on which the eggs are laid (K. L. Taylor, personal communication).

Formation of the venom gland complex of *Polistes*, with its greatly increased musculature and invaginated secretory tissue, marks another major advance in structural evolution. It is related to a diversion of function in the Vespoidea from paralysing the prey on which the eggs are laid to killing it as food for the developing brood.

Degenerative morphological changes appear in the venom glands of advanced members of both the Apidae and Formicidae. The glands are well developed in the primitive Bombini, in the Apini their secretion is used for defence only, and they become degenerate in the Meliponini (stingless bees), where the defence function is provided for in other ways (Kerr and de Lello 1962). In the primitive Formicidae venom is produced both for offense and defence (Cavill, Robertson, and Whitfield 1964). In the transition group Myrmicinae, in which there is a basic habit change from animal to plant foods, venom may be used defensively only, or be turned to some secondary purpose [*Atta texana*: Moser and Blum (1963)]. It reaches peak development as a defensive secretion among the Formicinae, while in the Dolichoderinae the glands producing venom tend to degenerate and other glands develop to take over the defence function.

(ii) *Dufour's Gland*

Although in the case of the venom glands it is possible to relate major changes in structure to changes in function, indicated by habits, it is more difficult to make deductions concerning Dufour's gland, since its functions are obscure. Earlier views suggest as possibilities that in the Formicidae its secretion may be a sting lubricant, a necessary ingredient of venom, or a means of neutralizing any acid poison adhering to the insect's own body (Wheeler 1913). The first of these opinions has persisted to the present day (Wigglesworth 1965) and studies on the chemical nature of Dufour's secretion in a number of Formicidae now demonstrate that its major constituents are hydrocarbons (Cavill and Williams 1967) whose function may well be that of lubrication.

The present research shows that Dufour's gland, like the venom glands, is represented throughout the Hymenoptera, and from group to group evolves to a phase of maximum structural development, then tends to degenerate and may finally be lost. It is to be expected that its functions should follow a similar pattern of development and change.

Dufour's gland secretion may function first as an egg lubricant, since its oily nature has been noted and its opening into the vagina traced in *Sirex* (Symphyta), and also in *Rhyssa* and *Megarhyssa* (Parasitica).

The fact that in certain Aculeata the position of the opening of Dufour's gland is carried away from the egg passage to within the sting bulb suggests that here it functions as a sting lubricant. This view is supported by the peak development of the gland in the Scoliodea, Sphecoidea, and Vespoidea, in which the use of the sting is brought to maximum efficiency.

Dufour's gland is well developed in primitive, stinging Formicidae, such as the Myrmecinae and Ponerinae, where it may perhaps retain some function as a sting lubricant. Consistent with this, in *Myrmecia gulosa* the diameter of the opening of Dufour's duct is approximately only one-twentieth that of the venom duct opening, so that no more than a trace of secretion can be released at any one time. Certainly Dufour's secretion must play some primary part in the process of venom ejection in *M. gulosa*, since its release is controlled by a branch of the same nerve from the terminal ganglion of the ventral nerve cord as controls the release of venom. In highly evolved Formicidae, where Dufour's gland is likely to be diverted to new functions (Wilson 1959; Maschwitz 1964), it may come to overshadow the venom glands in size (Fig. 12).

This then is the overall picture, deduced from present knowledge, of the purpose to which Dufour's gland secretion may be turned in the Hymenoptera. It is likely to function primarily as an egg lubricant in the Symphyta and Parasitica; secondarily as a sting lubricant, or some other adjunct to venom ejection, in the more generalized Aculeata; and thirdly, at the final evolutionary stage in advanced Aculeata, as an alarm substance, an odour trail marker, or some similar substance of non-reproductive significance.

(iii) *Sting Mechanism*

In wood-boring Hymenoptera the ovipositor has a saw-like structure which reaches peak development in the Siricoidea and Tenthredinoidea (Symphyta),

where it functions both as an egg-laying tube and as a probe to find favoured sites for oviposition. In the Parasitica it is used for the introduction of venom as well as for oviposition (Pampel 1914), and the shaft may become greatly extended in species like *Rhyssa persuasoria* (Ichneumonoidea), which are parasitic on wood-boring insects. In Vespoidea and Sphecoidea the principal function of the ovipositor, now become the sting, is to eject venom, the quantity produced being governed by the heavy muscles developed in the wall of the venom reservoir. In the Apoidea, in which the muscles of the reservoir wall are reduced or absent, valves are developed on the sting lancets which propel the venom along the sting (Snodgrass 1925). In primitive Formicoidea such as Myrmeciinae, both sting valves and reservoir wall muscles are well developed. In the highly evolved Formicinae, in which the sting has degenerated to a stage where it is no longer functional, the rudiments of its basal plates serve as a support for the nozzle structure through which a defence secretion is emitted, while its terminal spiracles provide the stream of air which enables the secretion to be ejected as a spray.

(iv) *Sting Glands*

Snodgrass (1925, 1956) described a mass of unicellular glands producing their secretion on to the outside of the quadrate plates of the sting in *Apis mellifera*, which he thought might lubricate the shaft of the sting when venom is being ejected. But Ghent and Gary (1962) showed that the glands produce an attractant which stimulates other bees to continue attacking at the original stinging site. It is the production of this attractant which appears to be responsible for the multiple stings sometimes inflicted on those handling bees. Maschwitz (1964) was unable to find the glands in *A. mellifera*, but with the techniques of the present work they are shown to be well developed indeed.

The current study demonstrates the occurrence of sting glands in super-families of Hymenoptera other than the Apoidea. They are recognized in relation to the triangular plate in the Formicoidea, in genera of Myrmeciinae and Ponerinae, and appear in the same region, as very large blocs of tissue, in the scoliid *Campsomeris* and the thynnid *Tachynomyia*. Presumably these glands do not stimulate stinging in non-social Hymenoptera such as the latter genera, which may well provide the answer to the problem of their primary function.

(c) *Conclusion*

In conclusion, some of the more general implications of the present study may be summed up as follows. The invagination into the venom reservoir of the zone of gland tissue in which the final synthesis of venom takes place is recognized as a widely adopted mechanism whereby the insect obtains additional protection against the toxicity of its own secretion. With this mechanism the gland tissue is doubly insulated from the remainder of the insect's body, both by its own wall and the wall of the reservoir. Again, the excessively developed muscles in the venom reservoir wall of the Scoliidea and Vespoidea are seen to have evolved in response to the need for maximum control over the increasingly potent venom used against their large and active prey. The musculature in other groups does not attain the same level of development, and that which exists is gradually lost as it ceases to be needed for any similar purpose. Clear links, through representatives of the primitive

Myrmeciinae and Ponerinae, are also demonstrated between the bourreleted and pulvinate types of advanced formicoid venom apparatus, whose relationship was previously little comprehended. Perhaps most important of all, grounds for considering the female accessory reproductive gland complex in the Symphyta, which includes both mucilage- and lipid-producing, but no toxin-producing elements, to be homologous with the venom apparatus of the higher Hymenoptera, are more firmly established in view of newly determined characteristics of the opening of the main exit duct, and because the early appearance of an Apocrita-type venom apparatus is now recognized in the transitional symphyte genus *Orussus*.

Considerable information is already available on the adult structure of the venom apparatus in various groups of Hymenoptera. But the overall pattern by which it has evolved could be defined more clearly by investigating the ontogeny of the apparatus, particularly in species representing primitive and transitional sections of the order. Justification for such studies lies not only in their biological importance, but also in the bearing they may have on our understanding of the pathways of chemical evolution followed by venom apparatus secretions.

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CORRIGENDUM

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The diagrams on pp. 551 and 561 should be interchanged but not the figure captions.